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IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: HEGEDUS, Lajos et al

Application No.: 09/299,562

Group:

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Examiner:

For: PHARMACEUTICAL COMPOSITIONS CONTAINING PLASMA PROTEIN

L E T T E R

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

August 5, 1999
3347-0101P

Sir:

Under the provisions of 35 USC 119 and 37 CFR 1.55(a), the applicant hereby claims the right of priority based on the following application(s):

Country	Application No.	Filed
HUNGARY	HU P 97 01554	09/18/97

A certified copy of the above-noted application(s) is(are) attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. 1.16 or under 37 C.F.R. 1.17; particularly, extension of time fees.

Respectfully submitted,

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ELSŐBBSÉGI PÉLDÁNY

1997 SZEPT 18.

Plazma protein tartalmú gyógyszerkészítmény.

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P-9727

PHARMACEUTICAL COMPOSITIONS CONTAINING PLASMA PROTEIN

The present invention is related to a method for delivery in therapeutic use of pharmacologically active compounds with poor water solubility and substantial binding to plasma proteins. More particularly it is related to water-soluble compounds and pharmaceutical formulations mainly for parenteral use, methods for their preparation and use; said compound and formulation containing

- a therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma proteins (in the following "the active ingredient") and
- a plasma protein fraction in controlled aggregation state and
- optionally further pharmaceutically acceptable additive(s) - such as water, stabilizer(s), protein aggregation controllers(s);

in the water-containing solutions of which the said active ingredient and the protein fraction are bound by way of non-covalent bonds and in the solid state forms of which the said active ingredient and the protein fraction may be bound by way of non-covalent bonds. Homogeneous solid state compounds of the invention are water-soluble and can be used parenterally or can be used to prepare parenteral pharmaceuticals.

It is well known that some biologically active compounds possess potent therapeutic activity but could never demonstrate their benefit because of their poor solubility in aqueous media. Some of them were never ever formulated while a few did not reach but the stage of the "phase I" clinical development. Some of them appears in "hardly biocompatible" formulations of relatively high toxicity caused by the materials used for formulation. A typical example for this is represented by the groups of taxones specifically paclitaxel which is a potent cytostatic the application of which however is reduced because of the toxicity of its formulation in Klucel : tween 80 or Klucel and diluent 12, a 1:1 mixture of cremaphor : ethanol. [Cancer Chemotherapy and Pharmacology (1994) 34:465-

471; Journal of the National Cancer Institute (1990) 1247-1259]. Cremaphor (polyoxyethylated castor oil) has inherent toxicity, causing vasodilatation, lethargy, hypotension etc.

Thus there is a need to solve the problem whereby therapeutically valuable water-insoluble products can be administered in water-soluble form to the patient in need to be treated with said active ingredients. The aim of this invention is to meet this requirement concerning practically water-insoluble active ingredients having a substantial binding activity to plasma proteins.

The present invention is based on the recognition that binding the active ingredients to adequate proteins before administration with non-covalent bonds presents a delivery system for the administration of the poor water solubility active ingredients. Using freeze drying solids can be produced which are re-dissolved in water whereby biocompatible, clear aqueous solutions are obtained which are suitable for parenteral administration. The invention presents a means to administer the desired water-insoluble active ingredients without introducing the toxic elements and in certain cases in a considerable more efficient dose than before.

Definitions used throughout this application which are henceforth not repeated:

R^1 represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide;

R^2 represents hydrogen or any acyl group preferably acetyl;

Low water-solubility means that the solubility in water at room temperature $< 1.10^{-7}$ M;

Substantial binding affinity to plasma proteins means that >90% of the ingredient is bound to the proteins in aqueous medium in spontaneous equilibrium at room temperature.

One object of the invention is a water-soluble human pharmaceutical formulation mainly for parenteral use containing a therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma

proteins and a human plasma protein fraction in controlled aggregation state.

Another object of the invention are water-soluble veterinary pharmaceutical formulations mainly for parenteral use containing a therapeutically active compound having low aqueous solubility and a substantial binding affinity to animal plasma proteins in controlled aggregation state.

The human or animal plasmas which can be present in the compositions according to the invention and accordingly can be used in the methods to prepare the compounds and compositions can be any of the naturally occurring protein fractions such as serum albumin, an immunoglubulin, glycoprotein, interferon and/or interleukin.

The practically water-insoluble active ingredients according to the invention comprise a wide range of compounds whereby the only limitation is that they have to show a substantial affinity to the plasma protein which is selected to be used. Examples for active ingredients include the following groups of therapeutic agents: a cytostatic such as a taxonoide, antibiotic, vitamin, antiinflammatory, analgesic, anticonvulsant, immunosupressant, antiepileptic, anxiolytic, hypnotic, antifungal agent, anticoagulant, lipid peroxidase inhibitor, coronary vasodilator, antiarrythmic agent, cardiotonic, urocosuric, antithrombotic, steroid hormone (progestogen, androgen, testogen) and/or photosensibilizer.

According to the invention some of the active ingredients which have poor water-solubility and have a substantial binding affinity to plasma proteins are enlisted in the following without the aim to restrict the scope of protection to these active ingredients:
a taxonoide, an adriamicine analogue, niflumic acid, apazone, suprofene, cyclosporine, tacrolimus, carbamazepine, phenytoin, valproic acid, phenobarbital, clonazepam, bromazepam, oxazepam, diazepam, flunitrazepam, amphotericin B, ketoconazole, dicumarol, warfarin, tirilazad, dipyridamole, disopyramide, digitoxine, sulfinpyrazone, progesterone, testosterone, ketochlorin.

A preferred embodiment of the invention consists in a formulation as described above containing a taxonoid of the general formula I.

Another preferred embodiment according to the invention contains paclitaxel and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other human plasma protein fraction.

It is clear from the above explanations that the invention covers the pharmaceutical formulations as above both in the solid states and also in the form of the aqueous solutions.

The plasma proteins used are preferably in a stabilized or controlled aggregation state. The aim is to avoid such aggregation of the proteins which would inhibit optimal binding of the active ingredient actually used. The unwanted aggregation of the proteins can be controlled by the presence of other molecules capable to occupy some or all of the binding sites on the macromolecules involved in the aggregation so as to avoid multiple protein - protein association.

One of the simplest aggregation controlling agent is water. Using the proper amount of water unwanted aggregation may be inhibited.

According to a preferred embodiment of the invention the compounds and compositions may contain as additive a protein aggregation controller or stabilizer and/or solution stabilizing auxiliary additive. Examples for such additives are the following: water, sodium chloride, a buffer, a poly-alcohol such as glycerol, a water-soluble sugar derivative preferably mannitol, sorbitol and/or dulcitol and others.

A further embodiment of the present invention includes the processes for the preparation of the pharmaceutical formulations as well as the compounds disclosed above comprising

i) dissolving the therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma proteins

("the active ingredient") in a water-miscible, pharmaceutically acceptable solvent and

ii) admixing said solution with the aqueous solution of a plasma protein fraction in controlled aggregation state containing optionally

iii) a further pharmaceutically acceptable auxiliary additive - preferably a protein aggregation controller or solvent stabilizer -

whereby a true solution is obtained containing the said active ingredient and the said protein fraction bound together by way of non-covalent bonds;

b) evaporating to dryness the solution *in vacuo* while freezing (lyophilization) whereby a homogeneous, solid state, water-soluble product is obtained containing the active ingredient and the plasma protein fraction;

c) optionally dissolving the solid product in water whereby a clear, liquid composition is obtained which is suitable for therapeutical administration and

d) optionally transforming this product into a parenteral formulation for direct use.

The proper solvent to be used to dissolve the active ingredient according to step a) above should have the following properties:

- it should be capable to completely dissolve the active ingredient in its mixture with water and
- its mixture with <50% of water should not denaturalize the protein employed.

Before selecting the proper solvent for the active ingredient and the protein selected the adequate solvent has to be determined routinely on the basis of the above. It is adequate to use solvents where mixtures containing >50% of water are still capable to dissolve the active ingredient.

Preferred solvents which can be used for step a) of the above process are for example an aliphatic $C_{(2-4)}$ monoalcohol or polyalcohol, preferably 70 - 100% ethanol.

When preparing the solution containing the protein an aggregation controller and/or solution stabilizer is preferably added. Such additives include a further or optimal amount of water. They also include agents capable to partially occupy some of the binding sites of the protein to avoid aggregation such as any of the following agents: sodium chloride, a buffer, a poly- alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol, dulcitol.

When selecting the optimal conditions in the case of any active ingredient the optimal binding properties and corresponding aggregation properties have to be determined by routine measurements. In the examples below we disclose the full method of such determinations.

According to a preferred embodiment of the invention the compounds and compositions used in step a) paclitaxel and a component of the natural plasma such as serum albumin, an immunoglubulin, glycoprotein, interferon and/or interleukin are used.

A further preferred embodiment of the invention consists in a homogeneous, solid, water-soluble product consisting of a taxonoide of the general formula I and a plasma protein fraction where the active ingredient and the plasma protein fraction can be in a non-covalent binding.

A preferred embodiment of the invention consists in a homogeneous, solid, water-soluble product consisting of paclitaxel and human serum albumin where the active ingredient and the plasma protein fraction can be in a non-covalent binding.

A further embodiment of the invention comprises the method of use of the compounds and compositions according to the invention for treatment of human or veterinary patients. The method consists in administering to a patient in need of a treatment with the active ingredient an effective dose of the

composition according to or prepared according to the invention. The doses that have to be applied depend on the active ingredient as well as on the protein used.

The compounds, compositions and methods of the invention present the following advantages:

- it becomes possible to avoid the use of biologically incompatible vehicles, to diminish or totally avoid dose limiting side effects, related to such components like toxic solvents, surface-active agents, emulsifiers and the like
- the use of plasma protein fractions as drug vehicles presents no adverse effects - to the contrary they may improve the tolerance of the patients e.g. in the case o chemotherapy
- in desired cases the applied dose can be increased as compared with the drugs now marketed containing biologically incompatible components and having dose limiting toxicity presenting thus a possibility to improve the overall outcome of therapy.

The present invention is illustrated in a more detailed manner in the following examples.

Examples

I. Preparation and Chemical Assays

Example I.1

The 20% (3.08×10^{-3}M) solution of human serum albumin in controlled aggregation state and the 1 mg/ml (1.17×10^{-3}M) solution of paclitaxel in absolute ethanol were admixed in 4:1 ratio and stirred obtaining a clear solution.

a) Ultrafiltration

A 1ml sample of the solution was filtered through an ultrafiltration membrane (cut off limit >30000) and paclitaxel was determined in the ultrafiltrate fraction. The sample loaded onto the ultrafiltration unit contained 15% of the total amount of paclitaxel. The measurable amount of paclitaxel in the ultrafiltrate fraction was due to the presence of ethanol (20%) in the solution. Measuring the paclitaxel concentration in the unfiltered solution 100% was recovered in unchanged form.

b.) Lyophilization

1 ml of the above solution was lyophilized. After lyophilization the solid residue was dissolved in 1.00 ml of distilled water, giving a clear solution. Measuring the paclitaxel concentration of this solution no paclitaxel was found in the water phase, but 100% was recoverable from the albumin fraction.

The assays for the determination of paclitaxel were done by HPLC with UV spectroscopy.

c) Assay for the determination of paclitaxel

A C-18 recersed phase HPLC chromatographic method was applied for the quantitative determination of paclitaxel from different solutions. The samples were injected into the HPLC system in $\geq$ 50% ethanol solution, preventing any precipitation of the substance.

The binding of the substance to plasma proteins was determined after 15 min equilibration at $8\pm2^{\circ}C$.

The distribution of the substance would be measurable after ultrafiltration through an appropriate membrane (cut-off must be > than the Mw of the protein), determining the substance concentration in the ultrafiltrate fraction (represents the unbound), and in the prefiltered solution, releasing the bond part with denaturation of the protein (represents the total). To the denaturation of the protein, and release the bond fraction, precooled ($8\pm2^{\circ}C$) abs. ethanol was used in 1:1 ratio.

The exact concentration values and amounts were calculated considering the dilution factor.

Examples I.2. to I.18.

The solution of human serum albumin in the concentration range of 20% (3.08×10^{-3}M) to 0.02% (3.08×10^{-6} M) was combined with the solution of paclitaxel in absolute ethanol in the concentration range from 20 mg/ml (2.34×10^{-2}M) to 0.01 mg/ml (1.17×10^{-5}M), obtaining always clear solutions.

Measuring the paclitaxel concentration bound to the albumin the maximum molar ratio of 17:1 for paclitaxel: albumin was achieved.

Details are presented in Table I.

All measurements were performed three times and the calculated results are averaged.

Table I.

$[T]_T$ (mM)	[HSA] (mM)	$n(T_B)/n(HSA)$	$n(T_B)/n(T_T) \times 100\%$
0.2342	2.410	0.093	97.4
0.2342	1.205	0.177	93.2
0.2342	0.602	0.346	91.0
0.2342	0.301	0.648	85.2
0.2342	0.121	1.545	81.2
0.2342	0.0602	3.125	82.1
0.2342	0.0241	5.662	59.5
0.2342	0.0121	4.948	26.0
0.2342	0.00602	5.823	15.3
0.2342	0.00241	10.419	11.0
0.2342	0.00121	14.367	7.6
0.2342	0.000602	12.370	3.3
4.6843	0.121	4.135	10.9
2.3421	0.121	8.401	44.2
1.1711	0.121	4.585	48.2
0.4648	0.121	2.864	75.3
0.1171	0.121	0.765	80.4

Legend:

$[T]_T$	total paclitaxel concentration after addition to HSA
[HSA]	concentration of human serum albumin (HSA)
$n(T_B)/n(HSA)$	number of moles of paclitaxel bound per mole of HSA
$n(T_B)/n(T_T)x100\%$	percentage of bound paclitaxel

Example 19.

The same method as above is used with animal serum albumin, immunoglobulines, glycoproteides, interferones or interleukines.

CLAIMS

1. A water-soluble pharmaceutical formulation mainly for parenteral use containing

   a) a therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma proteins (in the following "the active ingredient") and

   b) a plasma protein fraction in controlled aggregation state and

   c) optionally further pharmaceutically acceptable additive(s) - such as water, stabilizer(s), protein aggregation controllers(s);

   in the water-containing solutions of which the said active ingredient and the protein fraction are bound by way of non-covalent bonds and in the solid state forms of which the said active ingredient and the protein fraction may be bound by way of non-covalent bonds.

2. A water-soluble human pharmaceutical formulation according to claim 1 mainly for parenteral use containing a therapeutically active compound having low aqueous solubility and a substantial binding affinity to a human plasma protein fraction in controlled aggregation state.

3. A water-soluble veterinary pharmaceutical formulation according to claim 1. mainly for parenteral use containing a therapeutically active compound having low aqueous solubility and a substantial binding affinity to an animal plasma protein fraction in controlled aggregation state.

4. A formulation according to any of claims 1 to 3 containing a component of the natural - human or animal - plasma such as serum albumin, an immunoglubulin, glycoprotein, interferon and/or interleukin.

5. A formulation according to any of claims 1 to 4 for human administration containing a component of the natural - human plasma such as serum albumin, an immunoglubulin, glycoprotein, interferon and/or interleukin.

6. A formulation according to any of claims 1 to 5 containing as the water-insoluble active ingredient a cytostatic such as a taxonoide, antibiotic, vitamin, antiinflammatory, analgesic, anticonvulsant, immunosupressant, antiepileptic, anxiolytic, hypnotic, antifungal agent, anticoagulant, lipid peroxidase inhibitor, coronary vasodilator, antiarrythmic agent, cardiotonic, urocosuric, antithrombotic, steroid hormone (progestogen, androgen, testogen) and/or photosensibilizer.

7. A formulation according to any of claims 1 to 6 containing at least one of the following active ingredients: a taxonoide, an adriamicine analogue, niflumic acid, apazone, suprofene, cyclosporine, tacrolimus, carbamazepine, phenytoin, valproic acid, phenobarbital, clonazepam, bromazepam, oxazepam, diazepam, flunitrazepam, amphotericin B, ketoconazole, dicumarol, warfarin, tirilazad, dipyridamole, disopyramide, digitoxine, sulfinpyrazone, progesterone, testosterone, ketochlorin.

8. A formulation according to any of claims 6 and 7 containing a taxonoid of the general formula I - in the formula
   R^1 represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide
   R^2 represents hydrogen or any acyl group preferably acetyl.

9. A formulation according to any of claims 1 to 7 containing paclitaxel and human serum albumin, immunoglobulin, glycoprotein, interferon and/or interleukin or some other human plasma protein fraction.

10. A pharmaceutical formulation according to any of claims 1 to 9 having a solid state or having the form of an aqueous solution.

11. A pharmaceutical formulation according to any of claims 1 to 10 containing as additive an agent stabilizing the solution and/or the protein.

12. A pharmaceutical formulation according to claim 11 containing as solution and/or protein stabilizing agent anyone of the following: sodium chloride, a buffer, an alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol, dulcitol.

13. Process for the preparation of the pharmaceutical formulation according to any of claims 1 to 10 characterized by

a)

i) dissolving the therapeutically active compound having low aqueous solubility and a substantial binding affinity to plasma proteins ("the active ingredient") in a water-miscible, pharmaceutically acceptable solvent and

ii) admixing said solution with the aqueous solution of a plasma protein fraction in controlled aggregation state containing optionally

iii) a further pharmaceutically acceptable auxiliary additive - preferably a protein aggregation controller or a stabilizer -

whereby a true solution is obtained containing the said active ingredient and the said protein fraction bound together by way of non-covalent bonds;

b) evaporating to dryness the solution in vacuo while freezing (lyophilization) whereby a homogeneous, solid state, water-soluble product is obtained

containing the active ingredient and the plasma protein fraction;

c) optionally dissolving the solid product in water whereby a clear, liquid composition is obtained which is suitable for therapeutical administration and

d) optionally transforming this product into a parenteral formulation for direct use.

14. A process according to step a) of claim 13 characterized by using to dissolve the active ingredient a solvent having the following properties:

a) it is capable to completely dissolve the active ingredient in its mixture with water and

b) its mixture with <50% of water does not denaturalize the protein employed.

15. A process according to claim 14 characterized by using as the solvent an aliphatic $C_{(2-4)}$ monoalcohol or polyalcohol, preferably 70 - 100% ethanol.

16. A process according to step a) of claim 13 characterized by using as protein aggregation controller or stabilizer and/or solution stabilizing auxiliary additive any of the following agents: water, sodium chloride, a buffer, a poly- alcohol such as glycerol and/or a water-soluble sugar derivative preferably mannitol, sorbitol and/or dulcitol.

17. A process according to step a) of claim 13 characterized by using paclitaxel and a component of the natural plasma such as serum albumin, an immuno-glubulin, glycoprotein, interferon and/or interleukin.

18. A homogeneous, solid, water-soluble product consisting of a taxonoide of the general formula I - in the formula

R^1 represents tert. butyl-oxy-carboxylic acid amide or benzoyl amide,

R^2 represents hydrogen or any acyl group preferably acetyl -

and of a plasma protein fraction.

19. A homogeneous, solid, water-soluble product consisting of paclitaxel and human serum albumin.

20. Method of administering a water-insoluble pharmaceutical active ingredient having substantial plasma protein affinity characterized by administering to a patient in need of a treatment with said active ingredient an effective dose of the composition according to or prepared according to any of claims 1 to 19.

21. Method for parenteral delivery in therapeutic use of pharmaceutically active ingredients with poor solubility in water and substantial binding to plasma proteins characterized by administering to a patient in need of a treatment with said active ingredient an effective dose of the composition according to or prepared according to any of claims 1 to 19.

22. A product or method substantially as described in any of the examples.

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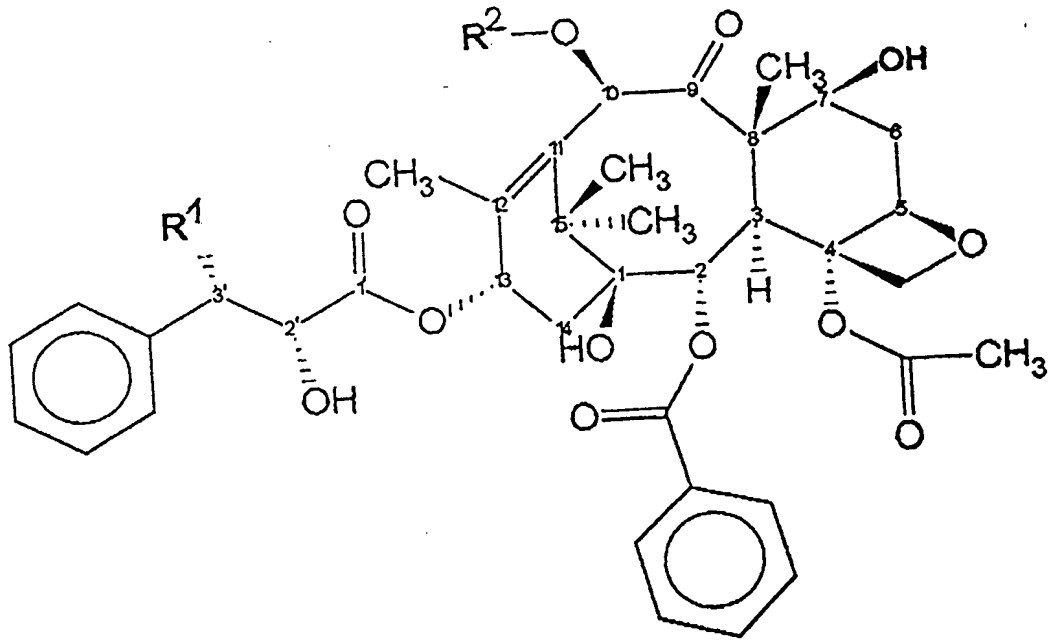

FIGURE 1

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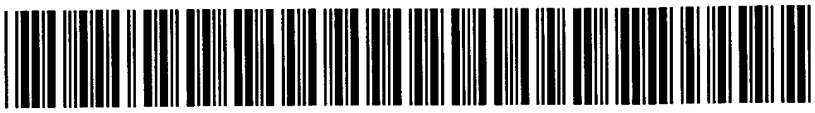

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